Journal of the American College of Cardiology
© 1999 by the American College of Cardiology
Published by Elsevier Science Inc.

Vol. 34, No. 2, 1999
ISSN 0735-1097/99/$20.00
PII S0735-1097(99)00199-0

Angiotensinogen Gene M235T Polymorphism Predicts Left Ventricular Hypertrophy in Endurance Athletes

Jouko Karjalainen, MD,* Urho M. Kujala, MD,† Anu Stolt, MD,† Matti Mäntysaari, MD,* Matti Viitasalo, MD,‡ Katariina Kainulainen, MD,‡ Kimmo Kontula, MD‡

Helsinki, Finland

OBJECTIVES We studied whether left ventricular mass in athletes associates with polymorphisms in genes encoding components of the renin–angiotensin system.

BACKGROUND Adaptive left ventricular hypertrophy is a feature of the athlete's heart. However, similarly training athletes develop left ventricular mass to a different extent, suggesting that genetic factors may modulate heart size.

METHODS We measured left ventricular mass by echocardiography in 50 male and 30 female elite endurance athletes aged 25 ± 4 (mean ± SD) years. Deoxyribonucleic acid samples were prepared for genotyping of angiotensinogen (AGT) gene M235T polymorphism, angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and angiotensin II type 1 receptor (AT1) gene A1166C polymorphism.

RESULTS The AGT gene M235T genotypes were significantly associated with left ventricular mass independently of blood pressure in both genders ($p = 0.0036$ for pooled data). TT homozygotes had greater mass compared with MM homozygotes in both men (147 ± 12 g/m vs. 132 ± 15 g/m, $p = 0.032$) and women (121 ± 12 g/m vs. 101 ± 13 g/m, $p = 0.019$). There was a gender difference in the relation between myocardial mass and AGT genotype, MT heterozygotes resembling MM homozygotes among women and TT homozygotes among men. The other studied gene polymorphisms were not associated with left ventricular mass.

CONCLUSIONS Angiotensinogen gene M235T polymorphism is associated with the variability in left ventricular hypertrophy induced by endurance training, with athletes homozygous for the T allele having the largest hearts. We found no association between ACE gene I/D or AT1 gene A1166C polymorphisms and left ventricular mass. (J Am Coll Cardiol 1999;34:494–9)
© 1999 by the American College of Cardiology

Cardiac hypertrophy usually follows an increase in workload imposed on the heart. In this process, mechanical, neural and endocrine mechanisms are important. However, individual myocardial responses to the workload are variable; for instance, there is a poor correlation between blood pressure and left ventricular (LV) hypertrophy in individual study participants (1). It has been observed that endurance athletes with similar training habits develop increased LV mass to different extents and that the amount of training accounts for only 11% of the variability in their LV mass (2). These findings suggest the potential importance of genetic factors in determining the heart size.

In endurance athletes, LV mass is on the average 45% greater than in matched control subjects (3). We hypothesized that a homogenous study population exposed to a strong, long-lasting and similar environmental myocardial growth stimulus, as is the case in young endurance athletes, could be an appropriate group for studying the possible genotypic modulation of increased LV mass. Recent experimental and clinical studies have suggested that the local cardiac renin–angiotensin system (RAS) may modulate cardiac growth. Synthesis of several important RAS components, including angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptor (AT1), has been shown to take place in myocardium and to be up-regulated during myocardial growth (4–6). We analyzed the associations of adaptive LV hypertrophy with three common allelic variations in these RAS components, namely the ACE gene insertion/deletion (I/D) polymor-

From the *Central Military Hospital; †Unit for Sports and Exercise Medicine, Institute of Biomedicine, University of Helsinki, and ‡Department of Medicine, University of Helsinki, Helsinki, Finland. This study was supported by grants from the Finnish Defense Forces, The Council of Health Sciences of the Finnish Academy, the Finnish Heart Foundation, the Sigrid Juselius Foundation and the Aarno Koskelo Foundation.

Manuscript received December 22, 1998; revised manuscript received March 2, 1999, accepted April 9, 1999.

Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
AGT	= angiotensinogen
ANCOVA	= analysis of covariance
AT1	= angiotensin II type 1 receptor
I/D	= insertion/deletion
LV	= left ventricular
PCR	= polymerase chain reaction
RAS	= renin–angiotensin system

phism (7–9), the AGT gene Met 235Thr polymorphism (9–11) and the AT1 gene A1166C polymorphism (12,13), chosen because of their suggested association with LV hypertrophy in selected clinical materials.

METHODS

Study population. We studied 80 elite endurance athletes from the Finnish national teams, 50 men and 30 women, aged 25 ± 4 (mean ± SD) years. The study group included long-distance runners, orienteers and cross-country skiers and triathlonists, all of whom gave their written informed consent for the study. Based on their training diaries, the subjects self-reported in the questionnaire their mean weekly volume (hours and minutes/week) of effective training including competitions. Most of the training was endurance training. None of the subjects used anabolic steroids or other drugs known to cause cardiac muscle hypertrophy. All participated in echocardiography to study the LV mass, geometry and filling. Blood pressure was obtained sphygmomanometrically in the supine position after 5 min rest. Peripheral blood was drawn and frozen for subsequent preparation of deoxyribonucleic acid for genotypic assays.

Echocardiography. Echocardiographic and Doppler studies were performed with an Acuson 128 instrument and V319 2.5- to 3.5-mHz transducer. To avoid including trabeculations (often prominent in an athlete's heart) in the wall thickness measurements, an integrated M-mode and two-dimensional study was done to determine interventricular septal and LV posterior wall thickness and end-diastolic cavity dimension as described in detail earlier (2). Left ventricular mass was calculated using the formula by Devereux (14):

$$\text{mass} = 0.8 \cdot [1.04 \cdot (\text{septal thickness} + \text{end-diastolic diameter} + \text{posterior wall thickness})^3 - \text{end-diastolic diameter}^3] + 0.6 \text{ g}.$$

Left ventricular mass was indexed to height. Concentricity of the myocardium was estimated by calculating the relative wall thickness using the formula:

$$\text{relative wall thickness} = (\text{septal thickness} + \text{posterior wall thickness})/\text{LV end-diastolic diameter}.$$

Measurements of LV diastolic filling velocities were obtained in an apical four-chamber view by positioning the pulsed Doppler volume sample about 1 cm below the mitral annulus. Early peak flow velocity (E) and peak atrial flow velocity (A) were measured and the ratio E/A calculated. All echocardiographic measurements were made by the same observer between 8:00 and 10:00 AM after a light breakfast, and obtained directly from the screen monitor with the aid of calipers and the instrument trackball.

Determination of AGT, ACE and AT1 genotypes. The ACE I/D polymorphism was determined according to Rigat et al. (15), and supplemented with confirmatory assays as suggested by Shanmugan et al. (16). The M235T polymorphism of the AGT gene and the A1166C polymorphism of the AT1 gene were assayed using the solid-phase minisequencing method (17). In the case of M235T, a polymerase chain reaction (PCR) product spanning the polymorphic site was generated using the biotinylated primer 5′-TCT GGA CTT CAC AGA ACT GGA T-3′ and the primer 5′-CTT GGA AGT GGA CGT AGG TGT-3′. The PCR products were captured in streptavidin-coated microtitration plate wells and rendered single stranded. The polymorphic nucleotide was detected in the captured deoxyribonucleic acid strand by extension of the "minisequencing" primer 5′-TGC TGT CCA CAC TGG CTC CC-3′ with a single ^{3}H-labeled deoxyadenosine triphosphate or deoxycytidine triphosphate. In the case of the A1166C polymorphism of the AT1 gene, a PCR product spanning the polymorphic site was generated using the biotinylated primer 5′-AAG CTT TTG TTC AGA GCT TTA G-3′ and the primer 5′-GTT CGA AAC CTG TCC ATA AAG-3′, whereafter the primer 5′-CAC TTC ACT ACC AAA TGA GC-3′ was used as the "minisequencing" primer (17).

Statistical methods. We tested our a priori hypothesis that polymorphisms in genes encoding the cascade of RAS (ACE I/D polymorphism, AGT M235T polymorphism and AT1 A1166C polymorphism) and LV mass were associated, using separate analysis of covariance (ANCOVA) models for each of the gene polymorphisms. Our basic ANCOVA models controlled for gender (between factor) as well as for age (covariate). All of our participants had competed in competitive sports since childhood; using age as covariate adjusted for years of training. Fisher least significant difference procedure test was used for post hoc multiple comparisons. For hypothesis testing the level of statistical significance was set at $p < 0.05$. Different analysis of variance and ANCOVA models as well as linear regression analysis were used to characterize in more detail the clinical significance of the statistically significant findings that supported the hypotheses. All analyses were performed

Table 1. Characteristics of Endurance Athletes

	Men (n = 50)	Women (n = 30)	p
Subject characteristic			
Age (yr)	24.7 ± 3.6	24.8 ± 4.1	NS
Height (cm)	182 ± 7	169 ± 7	<0.001
Weight (kg)	69 ± 7	58 ± 6	<0.001
Body mass index (kg/m^2)	20.8 ± 1.5	20.2 ± 1.8	NS
Heart rate (beats/min)	49 ± 7	51 ± 10	NS
Training (h/week)	10.3 ± 2.0	9.7 ± 2.2	NS
Systolic blood pressure (mm Hg)	129 ± 12	120 ± 9	<0.001
Diastolic blood pressure (mm Hg)	74 ± 10	75 ± 9	NS
Echocardiographic left ventricular data			
End-diastolic diameter (mm)	54.6 ± 3.0	50.8 ± 3.0	<0.001
Septum (mm)	11.9 ± 1.0	9.6 ± 0.8	<0.001
Posterior wall (mm)	11.1 ± 1.0	9.4 ± 1.0	<0.001
Length (mm)	100 ± 7	90 ± 5	<0.001
Mass (g)	255 ± 32	176 ± 29	<0.001
Mass/height (g/m)	140 ± 17	104 ± 16	<0.001
Relative wall thickness	0.42 ± 0.05	0.37 ± 0.04	<0.001
Filling velocity index (E/A)	2.5 ± 0.7	2.3 ± 0.5	NS

Numbers are mean ± SD.
E/A = early peak flow velocity/peak atrial flow velocity.

with the Statistica statistical package for Windows (Release 5, StatSoft, Tulsa, Oklahoma, 1995).

RESULTS

Characteristics of male and female athletes. Characteristics of the athletes and data on the echocardiographic measurements in men and women are summarized in Table 1. On average, indexed LV mass was 36% greater in male (140 ± 17 g/m) than in female (104 ± 16 g/m) athletes. For comparison, LV mass measured by the same observer and method was 93 ± 20 g/m in 15 age-matched sedentary men (2) and 68 ± 10 g/m in 15 age-matched sedentary women.

Frequencies of RAS gene genotypes. The frequencies of the different genotypes for the three gene polymorphisms examined are given in Table 2. All genotypic proportions were in Hardy–Weinberg equilibrium. In the whole sample, the ACE gene D and I allele frequencies were 54% and 46%, and the AGT gene M and T allele frequencies were 56% and 44%. These numbers do not differ significantly from those found among 523 randomly selected Finnish individuals (18). The A and C allele frequencies for the AT1 polymorphism were 76% and 24%, respectively.

Association of AGT genotype with LV mass. There was a significant association between the AGT genotype and LV mass in both male and female athletes (p = 0.0036 for pooled data) (Table 3, Fig. 1). This association was mostly due to differences in LV wall thicknesses (Table 4). As analyzed by a post hoc least significant difference test, subjects homozygous for the T allele had more hypertrophy than those homozygous for the M allele (p = 0.0013); this finding was significant in both male (p = 0.039) and female (p = 0.019) athletes. There was a gender difference in the heterozygotes; MT men had a greater (p = 0.021) LV mass compared with the MM homozygotes but did not differ significantly from TT homozygotes, whereas MT women differed significantly (p = 0.0038) only from the TT and not from MM homozygotes (Table 3). When the analyses were repeated with systolic or diastolic blood pressure as covariate, the association between AGT genotypes and LV mass did not weaken. Relative wall thickness did not associate significantly with AGT genotypes, although greater myocardial mass was associated with a more con-

Table 2. Renin–Angiotensin System Genotypes in 80 Endurance Athletes

Gene	Genotypes			Total Number of Subjects
	MM	**MT**	**TT**	
AGT				
Number	24 (30%)	41 (52%)	14 (18%)	79*
Male/female	17/7	24/17	8/6	49/30
	DD	**DI**	**II**	
ACE				
Number	24 (30%)	38 (47.5%)	18 (22.5%)	80
Male/female	15/9	23/15	12/6	50/30
	AA	**AC**	**CC**	
AT1				
Number	45 (58.5%)	27 (35%)	5 (6.5%)	77*
Male/female	30/15	17/10	1/4	48/29

*For technical reasons, angiotensinogen (AGT) genotype could not be determined in one subject and angiotensin II type 1 receptor (AT1) genotype in three subjects.
ACE = angiotensin-converting enzyme.

Table 3. Left Ventricular Mass (g/m) in Different Genotypes

	Genotypes and Left Ventricular Mass				
Gene	**MM**	**MT**	**TT**	**F**	**p**
AGT					
Men	132 ± 15	144 ± 18	147 ± 12	3.72	0.032
Women	101 ± 13	99 ± 15	121 ± 12	5.42	0.011
All*	123 ± 20	126 ± 28	136 ± 17	6.10	0.0036
	DD	**DI**	**II**		
ACE					
Men	145 ± 18	137 ± 15	141 ± 18	0.28	0.76
Women	103 ± 17	106 ± 17	100 ± 17	0.34	0.71
All*	129 ± 27	125 ± 22	127 ± 27	0.11	0.90
	AA	**AC or CC**			
AT1					
Men	143 ± 17	135 ± 16		1.79	0.19
Women	102 ± 16	105 ± 17		0.19	0.66
All*	130 ± 26	122 ± 22		0.34	0.56

*Gender and genotype as between-group factors. Abbreviations as in Table 2.

centric myocardial geometry ($r = 0.55$, $p < 0.0001$ for pooled data). Left ventricular filling velocities were not affected by the AGT gene polymorphism. There were no differences in body characteristics, volume of training or blood pressures between the athletes with different AGT genotypes (Table 4).

According to linear regression analysis, gender alone explained 60% of the variation in indexed LV mass. When men and women were considered separately, age and volume of training (mean training hours/week during the year before the examination) combined explained 9% and 15% of this variability, respectively. When AGT polymorphism was added to the model, the cumulative explanation rate increased by 13% (to 22%) in men and by 20% (to 35%) in women. Adding body mass index to the model did not significantly increase the explanation rate, obviously because it was low in all athletes.

Associations of the ACE and AT1 genotypes. Because only one man and four women had the AT1 gene CC homozygote, we compared AA homozygotes with the combined group of AC heterozygotes and CC homozygotes. Left ventricular mass, geometry or filling were not associated with the ACE or AT1 genotype (Table 3).

DISCUSSION

The novel result in this cross-sectional study was the strong association of adaptive LV hypertrophy with AGT gene M235T polymorphism. The finding was consistent in both men and women athletes, with those homozygous for the T allele having the greatest LV mass. In contrast, the ACE gene I/D genotypes and the AT1 gene A1166C genotypes were not significantly associated with LV mass.

Angiotensinogen polymorphism and LV hypertrophy. The M235T polymorphism of the human AGT gene was initially suggested to be associated with variation of serum AGT concentration and risk of essential hypertension (19). Subsequent studies have produced contradictory findings (for review, see Corvol and Jeunemaitre [6]). A large survey in Finland failed to demonstrate any association between M235T polymorphism and elevated blood pressure (18).

In a heterogenous study population of hospitalized patients in Japan, subjects with the TT genotype were found to have a significantly greater LV mass compared with those with the M allele (10). In contrast, the AGT genotypes were not associated with cardiac dimensions in a twin study (11). In a recent large study of Finnish hypertensive and control subjects, Kauma et al. did not find a significant association between AGT or ACE genotypes and LV mass, although in control men there was a clear tendency to greater ventricular mass in the TT genotype (9).

The strong physiologic stimulus to myocardial growth in our study subjects had caused an average increase in the LV mass of 50% in male and 53% in female athletes, compared with nonathletic age-matched subjects. It is reasonable to claim that these circumstances would effectively unmask any genetically determined modulating mechanisms. In contrast to many earlier studies involving heterogenous study populations with multiple environmental and biologic influences on myocardial mass, our study group was healthy and normotensive, racially uniform, had a low body mass index and most important, all had markedly increased LV mass. Gender was the most important variable affecting cardiac mass, explaining 60% of its variation. However, LV mass in MM homozygotes differed from that in TT homozygotes in both male (by 11%) and female athletes (by 20%). Interestingly, there was a clear gender difference in the relation between myocardial mass and AGT genotype, MT heterozygotes resembling MM homozygotes among women and TT homozygotes among men. Thus our results suggest

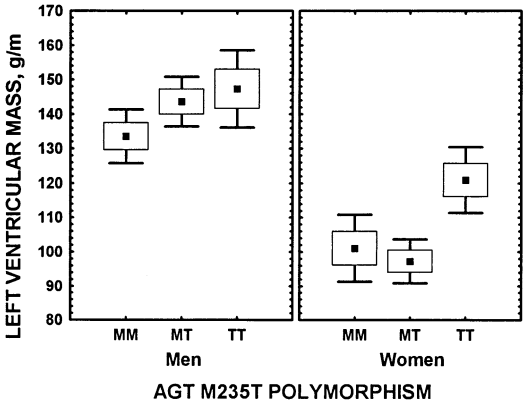

Figure 1. Left ventricular mass in endurance athletes with different genotypes of angiotensinogen (AGT) gene M235T polymorphism. **Solid squares** = mean; **boxes** = ±SE; **error bars** = 95% confidence intervals.

Table 4. Comparison of Athletes with Different AGT Genotypes

	AGT Genotype				
	MM	MT	TT	F	p
Age (yr)	25.5 ± 4.5	24.4 ± 3.3	24.6 ± 3.7	0.75	0.47
Height (cm)	177 ± 9	176 ± 9	177 ± 7	0.12	0.89
BMI (kg/m^2)	20.5 ± 1.3	20.6 ± 1.9	20.8 ± 1.5	0.36	0.70
Training (h/week)	10.4 ± 1.3	9.8 ± 2.2	10.5 ± 2.3	0.57	0.57
Systolic BP (mm Hg)	126 ± 12	125 ± 12	129 ± 11	1.08	0.34
Diastolic BP (mm Hg)	77 ± 11	74 ± 9	72 ± 9	1.93	0.15
LVEDD (mm)	53 ± 4	53 ± 4	54 ± 3	1.32	0.27
LV posterior wall (mm)	10.3 ± 1.0	10.4 ± 1.6	11.0 ± 1.3	4.06	0.021
E/A ratio	2.26 ± 0.58	2.51 ± 0.68	2.37 ± 0.39	1.63	0.20

Values are adjusted for gender.
AGT = angiotensinogen; BMI = body mass index; BP = blood pressure; E/A = early peak flow velocity/peak atrial flow velocity; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter.

that sex hormones may affect myocardial growth differently, depending on the number of T alleles the individual is carrying.

Other polymorphisms and LV hypertrophy. We found no significant associations between the ACE I/D polymorphism and estimates of LV dimensions. This seems to stand in contrast with the recent report by Montgomery et al. (20), who found that 10 weeks of training in military recruits increased LV mass more in carriers of the D allele than in those with the II genotype. However, in their study those with II genotype had the largest hearts at baseline. Our subjects had trained intensively for years, resulting in marked adaptive hypertrophy, which could partly explain the discrepancy between the studies. The reasons for the conflicting results in the many earlier studies concerning ACE genotypes and LV hypertrophy may involve differences and problems in genotyping assays, patient selection and ethnic background. In harmony with earlier studies (12,13), we did not find any association between LV hypertrophy and AT1 A1166C polymorphism.

Possible mechanisms of genotype–phenotype association. Recent studies point to the existence of local tissue RAS in the heart and many other tissues (4–6), which may function independently of the activity of the circulating RAS and even contribute to myocardial cell growth. In vitro and in vivo studies have suggested that mechanical stretch up-regulates the expression of the local RAS genes in cardiac myocytes, thereby initiating the positive feedback mechanism in load-induced cardiac hypertrophy (4,5). Expression of the AGT gene is increased during hemodynamic stress in rat's heart (21) and by stretch in cultured cardiac myocytes (4), creating conditions for an enhanced intracardiac angiotensin II formation. Angiotensin II has also been shown to increase myocardial protein synthesis (22), to promote cardiac myocyte growth (23) and to activate specific intracellular signaling cascades that result in cardiac hypertrophy (reviewed by Molkentin and Olson [24]). In healthy young adults, plasma angiotensin II level is associated with the size of LV independently of blood pressure (25). Collectively, all these data render the gene encoding AGT a likely candidate for a genetic factor influencing adaptive LV hypertrophy.

There is evidence that the concentration of AGT, instead of that of ACE, has the limiting role in generation of angiotensin II (6). Although there is no evidence that the M235T polymorphism directly affects the function or secretion of AGT, the concentration of AGT is about 13% to 20% higher in subjects carrying the 235T allele than in those without it (6). M235T polymorphism has been shown to be in linkage disequilibrium with a molecular variant in the proximal promoter of the AGT gene affecting the basal transcription rate of AGT (26), which also could explain the increased AGT levels in subjects carrying the 235T allele.

Study limitations. We have no information of our athletes' heart size in childhood before they adopted systematic and long-lasting training, which is a limitation of this study. Accordingly, there remains some uncertainty whether the AGT M235T polymorphism influenced the basal LV mass, or the exercise-induced increase in LV mass, or both. However, in other studies no significant differences in cardiac dimensions were found between different AGT genotypes in healthy nonathletic subjects (9,11). We studied only a limited number of athletes, and our results should be confirmed in a larger study of individuals with adaptive cardiac hypertrophy.

Conclusions. Our study provides evidence that variation in the AGT gene locus associates with LV mass independently of blood pressure in healthy individuals with adaptive cardiac hypertrophy.

Reprint requests and correspondence: Dr. Jouko Karjalainen, Central Military Hospital, Box 50, 00301 Helsinki, Finland. E-mail: jouko.karjalainen@pp.inet.fi.

REFERENCES

1. Levy D, Anderson KM, Savage DD, et al. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors: the Framingham Heart Study. Ann Intern Med 1988;108:7–13.
2. Karjalainen J, Mäntysaari M, Viitasalo M, Kujala U. Left ventricular mass, geometry, and filling in endurance athletes: association with exercise blood pressure. J Appl Physiol 1997;82:531–7.
3. Maron BJ. Structural features of the athlete heart as defined by echocardiography. J Am Coll Cardiol 1986;7:190–3.
4. Sadoshima J-I, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 1993;75:977–84.
5. Lee YA, Liang CS, Lee MA, Lindpaintner K. Local stress, not systemic factors, regulates gene expression of the cardiac renin-angiotensin system in vivo: a comprehensive study of all its components in the dog. Proc Natl Acad Sci USA 1996;93:11035–40.
6. Corvol P, Jeunemaitre X. Molecular genetics of human hypertension: role of angiotensinogen. Endocr Rev 1997;18:662–7.
7. Schunkert H, Hense H-W, Holmer SR, et al. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. N Engl J Med 1994;330:1634–8.
8. Kupari M, Perola M, Koskinen P, et al. Left ventricular size, mass, and function in relation to angiotensin-converting enzyme gene polymorphism in humans. Am J Physiol 1994;267:H1107–11.
9. Kauma H, Ikäheimo M, Savolainen MJ, et al. Variants of renin-angiotensin system genes and echocardiographic left ventricular mass. Eur Heart J 1998;19:1109–17.
10. Iwai N, Shimoike H, Ohmichi N, Kinoshita M. Angiotensinogen gene and blood pressure in Japanese population. Hypertension 1995; 25:688–93.
11. Busjahn A, Knoblauch H, Knoblauch M, et al. Angiotensin-converting enzyme and angiotensinogen gene polymorphisms, plasma levels, cardiac dimensions. A twin study. Hypertension 1997;29:165–70.
12. Castellano M, Muiesan ML, Beschi M, et al. Angiotensin II type 1 receptor A/C1166 polymorphism. Relationship with blood pressure and cardiovascular structure. Hypertension 1996;28:1076–80.
13. Hamon M, Amant C, Bauters C, et al. Association of angiotensin converting enzyme and angiotensin II type 1 receptor genotypes with ventricular function and mass in patients with angiographically normal coronary arteries. Heart 1997;77:502–5.
14. Devereux RB. Detection of left ventricular hypertrophy by M-mode echocardiography. Hypertension 1987;9 Suppl II:19–26.
15. Rigat B, Hubert C, Corvol P, Soubrer F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidylcarboxypeptidase 1). Nucl Acid Res 1992;20:251.
16. Shanmugan V, Sell KW, Saha BK. Mistyping ACE heterozygotes. PCR Methods Appl 1993;3:120–1.
17. Syvänen AC, Aalto-Setälä K, Harju L, et al. A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. Genomics 1990;8:684–92.
18. Kiema T-R, Kauma H, Rantala AO, Savolainen MJ. Variation at the angiotensin-converting enzyme gene and angiotensinogen gene loci in relation to blood pressure. Hypertension 1996;28:1070–5.
19. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. Molecular basis of human hypertension. Role of angiotensinogen. Cell 1992;71:169–80.
20. Montgomery H, Clarkson P, Dollery CM, et al. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. Circulation 1997;96:741–7.
21. Finkch M, Hellmann W, Ganten D, et al. Enhanced cardiac angiotensinogen gene expression and angiotensin converting enzyme activity in tachypacing-induced heart failure in rats. Basic Res Cardiol 1991; 86:303–16.
22. Geenen DL, Malhotra A, Scheuer J. Angiotensin II increases cardiac protein synthesis in adult rat heart. Am J Physiol 1993;265:H238–43.
23. Baker KM, Aceto JF. Angiotensin II stimulation of protein synthesis and cell growth in chick cells. Am J Physiol 1990;259:H610–8.
24. Molkentin JD, Olson EN. GATA4: a novel transcriptional regulator of cardiac hypertrophy. Circulation 1997;96:3833–5.
25. Harrap SP, Dominiczak AF, Fraser R, et al. Plasma angiotensin II, predisposition to hypertension, and left ventricular size in healthy young adults. Circulation 1996;93:1148–54.
26. Inoue I, Nakajima T, Williams CS, et al. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. J Clin Invest 1997;99:1786–97.